by HPLC. SW1353 and primary human chondrocytes obtained after total knee replacement surgery from patients with osteoarthritis (OA) were cultured in DMEM with 10% FCS. Cytotoxicity of different CSE concentrations was examined using MTT and BrdU assays. Quantitative real-time RT-PCR (RT-qPCR) was used to evaluate the effects of CSE on mRNA levels *of interleukin-1-beta (IL-1β), tumor necrosis factor-alpha (TNF-α), inducible nitric oxide synthase* (*iNOS*), *cyclooxygenase-2* (*COX-2*) and *matrix metalloproteinase-3 (MMP-3)*. The nitric oxide (NO) production was measured using Griess assay. The gelatinolytic activity of MMPs was determined by zymography. Transient transfection and luciferase assay was performed to study CSE action on NF-kappaB signalling.

Results: Dose-response experiments revealed that up to 20 μg/ml CSE did not impair viability of chondrocytes. CSE treatments (5-20 μg/ml) significantly suppressed the up-regulation of proinflammatory cytokine mRNA (*IL-1β* and *TNF-α*) in response to IL-1β (10 ng/ml) stimulation in SW1353 and primary chondrocytes ($p<0.05$). At the same concentrations, CSE inhibited the IL-1β induced up-regulation of *MMP3* mRNA and gelatinolytic activity of MMPs in a dose-dependent manner. A significant inhibition of NO production and *iNOS* mRNA expression was also observed in IL-1β stimulated primary chondrocytes pretreated with CSE ($p<0.05$). Cotransfections of the COX-2 promoter luciferase reporter plasmid together with p50 and p65 expression vectors enhanced the COX-2 promoter activity 3.4-fold in SW1353 cells. This p50/p65-mediated transactivation of the COX-2 promoter was dose-dependently down-regulated to basal levels by CSE.

Conclusions: These results indicate that CSE possesses potent anti-inflammatory activity in osteoarthritic chondrocytes. In particular, its action might involve the inhibition of NO production via *iNOS* downregulation as well as the inhibition of p50/p65-mediated COX-2 promoter activation.

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MODULATION OF CHONDROCYTE METABOLIC PATHWAYS BY NSAIDS AND THE CYCLOOXYGENASE-INHIBITING NITRIC OXIDE DONATOR (CINOD) NCX 429

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Purpose: Osteoarthritis (OA) progression is accompanied by a reduction of extracellular matrix and increased catabolism of collagen fibers and glycosaminoglycans in joints. Cyclooxygenase-inhibiting nitric oxide donators (CINODs) are novel anti-inflammatory compounds designed to provide balanced COX-1 and COX-2 inhibition while releasing nitric oxide (NO), an important modulator of vascular tone. In the cartilage, the role of NO is controversial, as it is a recognized marker of inflammation and a possible cause of chondrocyte loss, but also a potent immuno-modulating factor improving joint vascular perfusion. We investigated the effects of the CINOD NCX 429 and reference NSAIDs (naproxen and celecoxib) in stimulated adult rabbit chondrocytes, focusing on their catabolic and anabolic activities, as well as inflammatory parameters.

Methods: Primary cultures of adult rabbit (4-6 months) articular chondrocytes (RACs) were pre-incubated with test drugs (1-10 μM, 8 hours) before addition of IL-1β (10 ng/ml, 16 additional hours) in 5% O_2 conditions, to mimic joint limited perfusion. Levels of mRNA were determined by quantitative RT-PCR for assessing expression of extracellular matrix proteins (aggrecan and collagen type II) and matrix proteases [aggrecanases and matrix metalloproteases (MMPs)]. NO production was determined by measurement of nitrite/nitrate (NOx) using the Griess method. Type II collagen production was evaluated in the supernatant by Western blot analysis. To assess COX inhibition, prostaglandin E_2 (PGE_2) was measured by enzyme immunoassay in the supernatant of IL-1β stimulated chondrocytes (from 2 months-old rabbits) incubated with NCX 429 (1-100 μM).

Results: NCX 429 and naproxen did not show any impact on RAC viability. In IL-1β-stimulated RACs, NCX 429 induced a 2-fold increase in type II collagen expression, similarly to naproxen and celecoxib. NCX 429 stimulated type II collagen at both mRNA and protein levels. In addition, NCX 429 reduced IL-1β-stimulated expression of MMP-1 by ~ 50% and aggrecanase-1 and 2 expression (by ~ 50% and 40%, respectively), with similar efficacy to naproxen and celecoxib. Conversely, IL-1β-induced increase in MMP-3 and decrease in aggrecan expression were not prevented by NCX 429 or naproxen, whereas celecoxib inhibited MMP-3 expression by 40%. Interestingly, while naproxen and celecoxib increased IL-1β-stimulated NOx levels by ~ 50 and 30%, respectively, NCX 429 decreased NOx levels (by ~ 25%). Finally, NCX 429 inhibited IL-1β-induced PGE_2 biosynthesis in a concentration-dependent manner, indicating effective COX inhibition.

Conclusions: NCX 429, naproxen and celecoxib modulate IL-1β-induced expression of catabolic and anabolic markers in stimulated adult rabbit articular chondrocytes. Interestingly, the CINOD NCX 429 slightly reduces IL-1β-induced increase of endogenous NO synthesis, differently from the other NSAIDs, likely by inhibiting inducible nitric oxide synthase (iNOS) expression, as already reported for other CINODs. NO has been considered as potentially detrimental for chondrocytes; however, donation of low concentrations of NO from the CINOD NCX 429 does not seem to affect chondrocyte survival or to increase the expression of mediators involved in cartilage degeneration. CINODs may even inhibit endogenous overproduction of NO, and therefore may represent an interesting therapeutic approach for the treatment of osteoarthritis.

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EFFECTS OF RIBONUCLEINATE COMPONENTS OF OSTEOCHONDRIN^{R} S OSTEOARTHRITIS THERAPY ON FORMATION AND ACTIVITY OF HUMAN OSTEOCLASTS *IN VITRO*

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Purpose: To establish if the ribonucleinate components of the osteoarthritis natural drug, Osteochondrin® S (OST) affect the bone-resorbing functions and gene expression in osteoclasts.

Methods: Human osteoclasts were generated *in vitro* from culture of RANKL-M-CSF stimulated peripheral blood mononuclear cells for 17d. Cells were treated with 23.5-587.2 ng/mL OST or 0.2-5 mg/mL RNA components of connective tissues or yeast (as used to make OST) and their effects on osteoclast formation were assessed using a Tartrate-Resistant Acid Phosphatase (TRAP) stain. Cell were also grown on dentine slices and used to determine the effects on osteoclast bone resorption was The pit formation on dentine was determined by scanning electron microscopy. In separate experiments, real time PCR was used to determine mRNA expression of principal enzymes or signalling molecules governing osteoclast functions.

Results: OST and its RNA components showed approximately equivalent concentration-dependent inhibition of TRAP stained osteoclasts and associated reduction in pit formation on dentine (Fig. 1A–F).

OST was, however, more potent in preventing bone resorption than the individual RNAs. mRNA expression of calcitonin receptor (CTR) was reduced

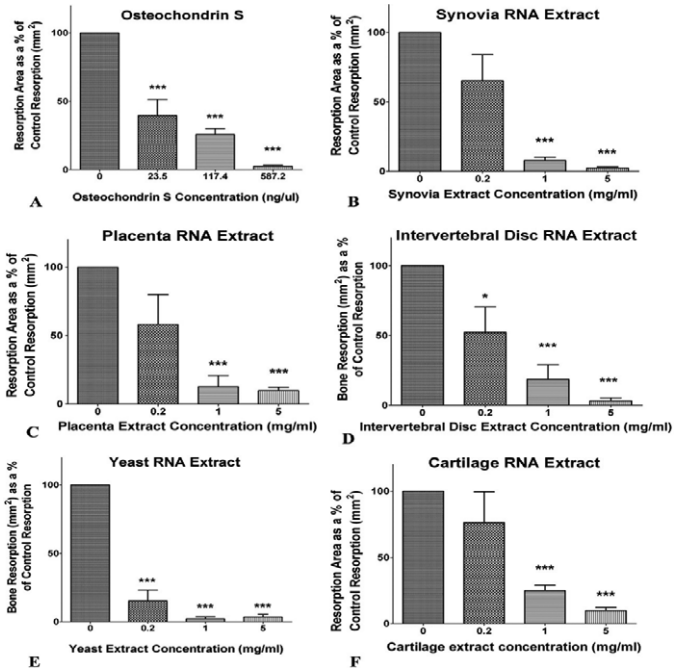

Figure 1A–F: Effects of OST and RNA components on osteoclast-mediated bone resorption. Statistical significance (***$p<0.001$; *$p<0.05$) - one-way ANOVA.